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SHORT COMMUNICATION

Chemically mediated burrow recognition in the Mexican tarantula *Brachypelma vagans* female

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Abstract Chemically mediated communication is common in spiders but has been poorly studied in burrowing tarantulas. This study aimed to determine whether chemical cues influence the behaviour of females of *Brachypelma vagans*, a Mexican species of tarantula, during encounters with previously inhabited burrows or with extracts from the silk of conspecific females. In laboratory choice tests, female tarantulas entered a burrow that had previously been inhabited by a conspecific female significantly more frequently than a burrow that had never been inhabited. The identity of the previous inhabitant also affected the number of spiders that chose to enter a burrow. Spiders were quicker to choose and enter a burrow previously inhabited by themselves than a burrow previously inhabited by a conspecific or a burrow that had not been previously inhabited. Hexane, methanol and dichloromethane extracts of conspecific silk elicited different responses from female tarantulas when extracts were placed on filter paper disks at one end of an experimental arena with a control filter paper disk, on to which the corresponding solvent alone had been pipetted, placed on the other end of the arena. Spiders showed the strongest responses to hexane extracts of silk, with a significant preference to move towards the hexane extract and a significantly greater period of time spent in proximity to the hexane extract compared to the control disk. Overall and in contrast to expectations, tarantulas were most strongly attracted to the cues left by other conspecific females. As encounters between *B. vagans* females usually lead to aggression and mortality of one of the participants, we conclude that chemical cues are not signals that are deliberately released by burrow-inhabiting females but may inadvertently escape and cannot be easily suppressed.

Keywords *Brachypelma vagans* · Chemical cues · Silk extraction · Intraspecific recognition

Introduction

Olfaction is considered to be an important ancestral means of communication in arachnids (Weygoldt 1977). Spiders use chemical cues for numerous purposes (Barth 2002), including prey detection (Jackson et al. 2005), aggressive mimicry (Gemeno et al. 2000), predator avoidance (Schonewolf et al. 2006), species discrimination (Persons and Lynam 2004; Roberts and Uetz 2004) and in the detection of conspecific males (Cross et al. 2007) and females (Clark et al. 1999). Most species of tarantula spiders have limited visual faculties, and intra-specific communication is achieved by vibratory signals (Redondo 1994), for example, during courtship (Quirici and Costa 2007). It has also been suggested that chemical cues may be important for intra-specific recognition prior to mating (Costa and Pérez-Miles 2002), but the role of such cues in

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inter-female communication has not been studied previously in tarantulas.

The Mexican tarantula *Brachypelma vagans* (Ausserer 1875) is a burrowing spider that is distributed from southern Mexico to Costa Rica. Field observations indicate that the females frequently locate and enter the burrows of conspecifics and then attack and consume the other female (Hénaut and Machkour-M'Rabet 2005). The aim of this study was to determine whether *B. vagans* females use chemical cues to detect burrows inhabited by conspecific females. We also discuss the likely costs and benefits to the sender or receiver of such chemical signals.

Materials and methods

Spiders were captured close to the Calakmul Biosphere Reserve, Campeche, Mexico (Machkour-M'Rabet et al. 2007) and were maintained individually in plastic boxes (5×13×8 cm) containing a dish of water, fed with living larvae of *Zophobas morio* (Coleoptera) and maintained at room temperature (24–28°C), ~75% relative humidity and 12:12-h photoperiod. All spiders were adult females, ~4 cm in length as measured from the anterior point of the cephalothorax to the posterior tip of the abdomen.

We tested burrow selection choices by female tarantulas in an experimental arena containing artificial burrows. All experiments were performed using clear plastic boxes (30×40×30 cm) containing a 20-cm-deep layer of clay soil (collected from close to the Calakmul Reserve), in which two vertical burrows were excavated in opposite corners at one end of the box (Fig. 1a). Each burrow measured 5 cm in diameter and 5 cm in depth and was constructed by hand while wearing plastic gloves.

A female tarantula was placed in a burrow and sealed inside with a plastic cover for 24 h, as this gave the spiders enough time to lay down silk and produce the chemical cues associated with an inhabited burrow. The plastic cover and tarantula were then removed, and the burrow was considered as having been marked. A spider was then placed at the opposite end of the experimental arena (Fig. 1a) and observed continuously for 30 min. The burrow chosen by the spider and the time between introduction into the arena and entry into a burrow were recorded. Results from spiders that did not initiate any locomotion behaviour within 30 min of the start of the experiment were not used in result's analyses.

There were four objectives:

1. To test the ability to differentiate between burrows previously inhabited by themselves and burrows previously inhabited by other conspecific female, one tarantula was tested at a time by placing it in an arena that contained two burrows. One burrow had been inhabited previously by the test spider and the other burrow had been previously inhabited by another conspecific female (Fig. 1a, test 1).
2. To test whether the tarantula was attracted to the physical or chemical cues of its own burrow, one burrow was inhabited by the test spider and the other burrow was not inhabited and was sealed with a plastic cover ("Blank" burrow). The test tarantula was then placed in the arena and allowed to choose between each kind of burrow (Fig. 1a, test 2).
3. To test whether tarantulas were attracted to the cues left at the burrow by another conspecific female, one burrow was inhabited by a female and the other burrow was left blank. A different conspecific female was then placed in the arena and allowed to choose between each kind of burrow (Fig. 1a, test 3).

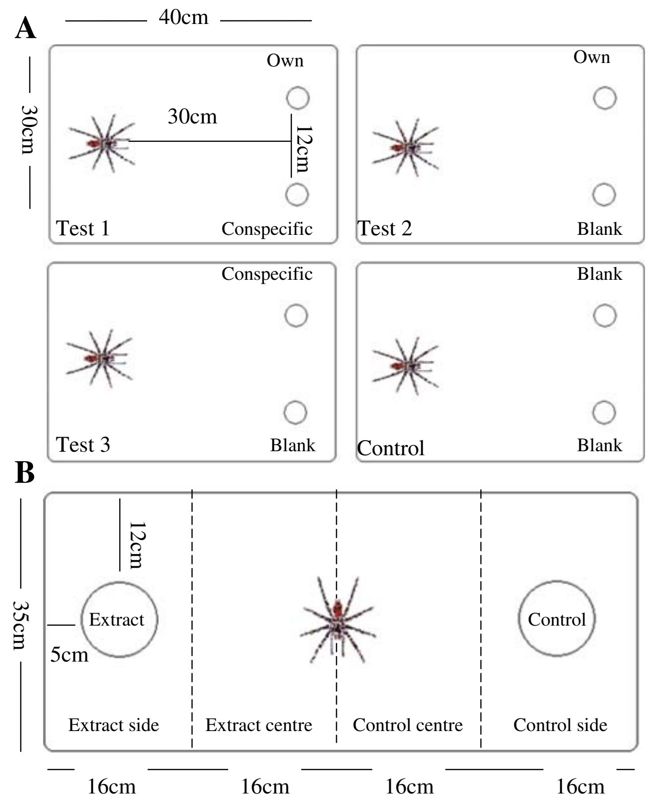

Fig. 1 Experimental arena designs for all behavioural observations on *B. vagans*. **a** Burrows were previously inhabited for 24 h by a tarantula or left empty (*Blank burrow*). Tests involved a choice between (1) a burrow previously inhabited by the tarantula (*Own*) or one previously inhabited by a different conspecific female (*Test 1*); (2) a burrow previously inhabited by the tarantula or a blank burrow (*Test 2*); (3) a burrow previously inhabited by a different conspecific female or a blank burrow (*Test 3*); (4) two blank burrows that had not been previously inhabited (*Control*). **b** Bioassay device to determine tarantula responses to solvent extracts of silk. The arena was classified into four zones according to the presence of a paper disk treated with solvent extract or a control disk treated with solvent alone

4. To ensure that burrow choice was not affected by factors other than the prior occupant of the burrow, a control test was carried out with two blank burrows. A tarantula was then placed in the arena and allowed to choose between these burrows (Fig. 1a, control).

After each experiment, the plastic box was cleaned with unperfumed detergent and water, rinsed with ethanol and allowed to dry. The soil was completely replaced on each occasion. For each of the four objectives, there were 30 replicates, each replicate being with a different spider.

To collect volatile compounds from silk, 12 females of *B. vagans* were individually placed in cylindrical glasses (12 cm tall, ∅8 cm) that had been previously washed with acetone and distilled water. Each glass was sealed with aluminium foil and left for 24 h, during which time spiders could deposit silk on the glass wall. They were then removed, and the silk was collected from each glass individually by wrapping it around a sterile Pasteur pipette. Extraction of the chemical compounds was performed immediately by exposing each silk sample to 200 µl of solvent for a 30-min period. Three different solvents were used: hexane, dichloromethane and methanol. The 200-µl volume was concentrated by nitrogen evaporation to a total volume of 5 µl and stored in a glass vial at 5°C until used.

For behavioural observations, a plastic box (63×35×22 cm) was used. The box was cleaned with acetone, and the floor was covered with clean white office paper before each trial. A 5-µl volume of each silk extract was pipetted onto the centre of a filter paper disk (∅10 cm, Whatman no. 2). The treated disk was then placed on the floor at one end of the experimental arena, and a control disk, on to which 5 µl of solvent alone had been pipetted, was placed at the other end of the arena (Fig. 1b). Separate controls were used for each of the solvents. We divided the arena into four zones of equal area: extract-side, extract-central, control-side and control-central. Two variables were determined for each solvent: number of crossings (total number of crossings between one zone and the next) and time (total time spent in each zone during each test). When the disks were in place, a single tarantula was gently introduced into the middle of the box and observed for 30 min. Observations using each of the three solvents were performed 15 times with different extracts and different individual spiders. Experimental spiders were never re-tested.

Statistical analyses

Frequencies of choices for each type of burrow were compared by the *G* test (Zar 1996). The times taken to reach a burrow were compared by Mann–Whitney *U* test. Only times involving direct paths (i.e., paths that did not cross themselves) were included in the analyses. In the experiment involving solvent extracts, the number of times that spiders crossed between zones of the arena and the time spent in each zone were compared by Friedman test for multiple dependent samples followed by Dunn test (Zar 1996).

Results

During behavioural tests, two tarantulas stayed immobile in test 1, five in test 2, seven in test 3 and none in the control.

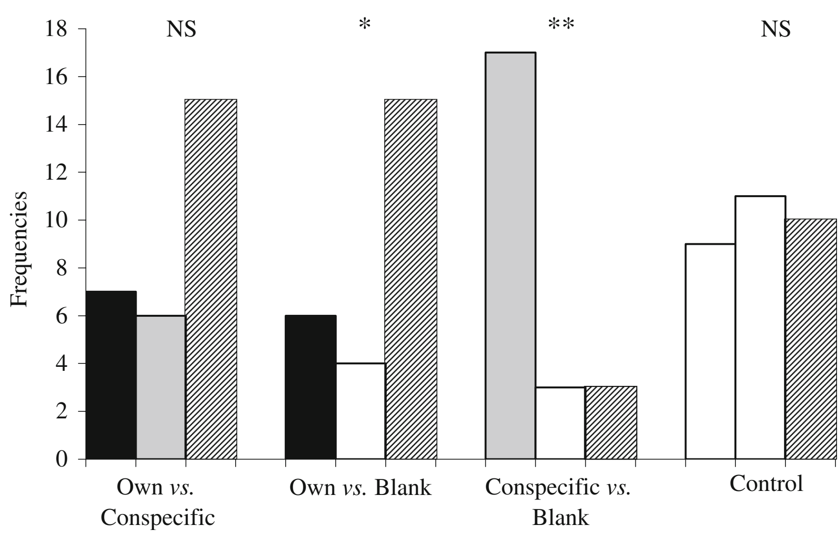

Fig. 2 Number of responses for all binary choices in the experiments involving choices of burrows that had been previously inhabited by *B. vagans*. *Black*, choice of own burrow, *gray* choice of conspecific burrow, *white* choice of a blank burrow, *hatched* no choice, *NS* not significant. *$P<0.05$, ***$P<0.001$, *G* test (see main text for statistical details)

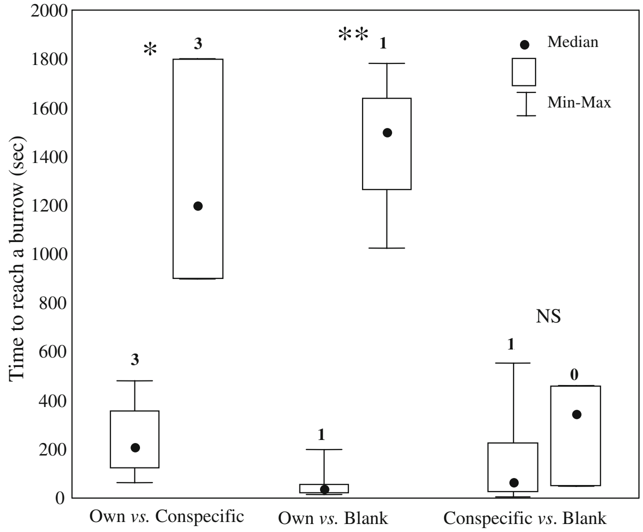

Fig. 3 Median values and interquartile ranges (25–75%) of times taken to reach a burrow in the burrow choice experiments. Only times involving direct paths were analysed. *Numbers above bars* indicate the number of indirect paths that were excluded from the analysis. No direct paths were observed in the control experiment. *NS*, not significant. *$P<0.05$, ***$P<0.001$, Mann–Whitney test (see main text for statistical details)

In the other replicates, not all individuals chose to enter burrows; but in all cases, tarantulas were observed physically exploring the experimental arena, so that all those individuals were included in the results as "no choice". Tarantulas showed no significant choice between their own burrow and a burrow previously inhabited by a different conspecific female (G=4.90, df=2, P=0.09; Fig. 2). When presented with a choice between its own burrow and a blank burrow, the majority of spiders choose to not enter either type of burrow (G=7.82, df=2, P=0.02). In contrast, tarantulas chose to enter a burrow previously inhabited by a different conspecific female significantly more frequently than a blank burrow (G=15.81, df=2, P<0.001). Tarantulas showed no significant choice when tested with two previously uninhabited burrows, i.e., the control (G=0.20, df=2, P=0.9). A dramatic difference was observed in the distribution of choices made in the second and third tests (Fig. 2). A test of independence on whether choosing versus not choosing was dependent on whether the burrow had been occupied by the test spider or by a different conspecific female was significant (G=12.93, df=1, P<0.001), indicating that the identity of the prior occupant was highly influential in the decision-making process.

The times taken to enter the burrow depended on the choice of burrows available (Fig. 3). Spiders were quicker to choose a burrow previously inhabited by themselves than a burrow previously inhabited by a conspecific (test 1; $U_{1,7}$=0, P=0.034) or a blank burrow (test 2; $U_{1,9}$=0, P=0.014). No significant differences were detected in the times spent in reaching each type of burrow in the experiment involving a choice between a conspecific burrow and a blank burrow (test 3; $U_{1,19}$=11, P=0.15). No direct paths were observed in the control test, and times to enter burrows were not subjected to analysis.

When silk extracts were used in trials, spider movement between each of the four zones of the experimental arena (Table 1) did not differ significantly when filter paper disks were treated with methanol ($\phi_3^2 = 5.832$, P=0.15) or dichloromethane ($\phi_3^2 = 0.441$, P=0.9) extracts of silk, whereas the presence of hexane extracts resulted in a significantly increased incidence of crossing into the zone in which the treated disk was located (extract side, $\phi_3^2 = 12.945$, P=0.005). How long tarantulas spent in each zone differed significantly for each solvent (methanol, $\phi_3^2 = 22.397$, P<0.001; dichloromethane, $\phi_3^2 = 12.837$, P=0.005; hexane, $\phi_3^2 = 27.716$, P<0.001). Spiders spent significantly more time in the zone surrounding the disk treated with a hexane extract of silk than in any other zone (Table 1), whereas the times spent in proximity to methanol and dichloromethane extracts were not consistently greater than the times spent in other zones of the arena.

Table 1 Mean (±SE) number of crossings between zones and mean (±SE) time spent in each zone of the experimental arena, as defined by the presence of a filter paper treated disk with tarantula silk extracted using different solvents or a solvent control

	Solvent	Zone of experimental arena				P value
		Extract side	Extract centre	Control centre	Control side	
Mean of crossings between zones	Methanol	6.8±1.0	5.7±1.0	5.2±1.2	5.5±1.2	0.1
	Dichloromethane	3.7±1.1	3.4±1.0	3.6±1.0	3.7±1.0	0.9
	Hexane	5.6±0.7 a	4.2±0.6 b	4.0±0.6 b	4.1±0.7 b	0.005
Time spent in each zone (s)	Methanol	1086±133 a	193±89 bc	87±19 c	432±86 ab	0.001
	Dichloromethane	642±180 a	181±112 b	136±73 b	967±183 a	0.005
	Hexane	1052±119 a	195±90 b	128±41 b	422±100 b	0.001

Freidman test, for each comparison, means labelled with different letters differed significantly (Dunn test)

Discussion

Cues associated with burrows that had been previously occupied by females of *B. vagans* affected the behaviour of other conspecific females. A resident female was allowed to lay down silk in the burrow, and it is known that spiders are often attracted to cues from the silk of conspecific individuals, a phenomenon known as sericophily (Hodge and Stoffer-Isser 1997). Chemical cues derived from solvent extracts of the silk of *B. vagans*, particularly extracts in hexane, were sufficient to elicit the attraction of conspecific females. This suggests that the principal compounds responsible for influencing the behaviour of *B. vagans* females are non-polar organic compounds comprising ten to 18 carbon atoms (Howard and Blomquist 2005). Carbon compounds of a similar size are known to be important as chemical cues, particularly as sex pheromones in other arthropods, including other spiders (Gaskett 2007). However, our findings seem to differ from better-known examples of chemical signals exchanged between conspecific individuals that are usually advantageous to both the sender and the receiver; sex pheromones and territorial markers are two well-studied examples of mutually beneficial signals. In the case of our study, the chemical cues associated with spider silk appear to be of no benefit to the sender of the signal. We would expect to find animals strongly attracted to the territorial signposts they left themselves, but we found instead that tarantulas were most strongly attracted to the cues left by other conspecific females. When *B. vagans* females encounter each other, aggressive interactions normally ensue (Hénaut and Machkour-M'Rabet 2005). This suggests that chemical cues are not signals that are deliberately released by the tarantulas, but rather these compounds may escape inadvertently from the burrow-inhabiting females and cannot be easily suppressed (see Clark et al. 1999 for a similar phenomenon in salticid spiders).

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References

Barth FG (2002) A spider's world: senses and behavior. Springer, Heidelberg

Clark RJ, Jackson RR, Waas JR (1999) Draglines and assessment of fighting ability in cannibalistic jumping spiders. J Insect Behav 12:753–766

Costa F, Pérez-Miles F (2002) Reproductive biology of Uruguayan theraphosids (Araneae, Mygalomorphae). J Arachnol 30:571–587

Cross FR, Jackson RR, Pollard SD, Walker MW (2007) Cross-modality effects during male–male interactions of jumping spiders. Behav Process 75:290–296

Gaskett AC (2007) Spider sex pheromones: emission, reception, structures, and functions. Biol Rev 82:27–48

Gemeno C, Yeargan KV, Haynes KF (2000) Aggressive mimicry by the bolas spider *Mastophora hutchinsoni*: identification and quantification of a major prey's sex pheromone components in the spider's volatile emissions. J Chem Ecol 26:1235–1243

Hénaut Y, Machkour-M'Rabet S (2005) Canibalismo y cleptobiosis en la tarántula *Brachypelma vagans*. Entomol Mex 4:30–32

Hodge MA, Storfer-Isser A (1997) Conspecific and heterospecific attraction: a mechanism of web-site selection leading to aggregation formation by web-building spiders. Ethology 103:815–826

Howard RW, Blomquist GJ (2005) Ecological, behavioural, and biochemical aspects of insects hydrocarbons. Ann Rev Entomol 50:371–393

Jackson RR, Nelson XJ, Sune GO (2005) A spider that feeds indirectly on vertebrate blood by choosing female mosquitoes as prey. Proc Natl Acad Sci USA 102:15155–15160

Machkour-M'Rabet S, Hénaut Y, Sepulveda A, Rojo R, Calmé S, Geissen V (2007) Soil preference and burrow structure of an endangered tarantula *Brachypelma vagans* (Mygalomorpheae: Theraphosidae). J Nat Hist 41:1025–1033

Persons MH, Lynam EC (2004) *Pardosa milvina* (Araneae, Lycosidae) spiderling movement in the presence of conspecific and heterospecific silk and excreta. J Arachnol 32:341–344

Quirici V, Costa FG (2007) Seismic sexual signal design of two sympatric burrowing tarantula spiders from meadows of Uruguay: *Eupalaestrus weijenberghi* and *Acnathoscurria suina* (Araneae, Theraphosidae). J Arachnol 35:38–45

Redondo T (1994) Comunicación: teoría y evolución de las señales. In: Carranza J (ed) Etología: Introducción a la Ciencia del Comportamiento. Universidad de Extremadura, Caceres, Spain, pp 255–297

Roberts JA, Uetz GW (2004) Chemical signalling in a wolf spider: a test of ethospecies discrimination. J Chem Ecol 30:1271–1284

Schonewolf KW, Bell B, Rypstra AL, Persons MH (2006) Field evidence of an airborne enemy-avoidance kairomone in wolf spiders. J Chem Ecol 32:1565–1576

Weygoldt P (1977) Communication in crustaceans and arachnids. In: Sebeok TA (ed) How animals communicate. Indiana University Press, Bloomington, pp 303–333

Zar JH (1996) Biostatistical analysis, 3rd edn. Prentice-Hall, New York